[MS5-P03] Crystal Structure of a Novel Enoate Reducase in the Fatty Acid Metabolism.

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In a living organism, fatty acids exist as the constituents of neutral and polar lipids, the covalent attachments to distinct eukaryotic proteins, and the parts of eucaryotic second-messenger molecules and serve many essential functions.[1,2] They can be remodeled by elongation, insertion or removal of double bonds, or covalently bind to proteins in a living organism. Several metabolic pathways have been discovered and studied to investigate the mechanism of the reactions and the regulation of fatty acids. Recently, a novel saturation pathway of linoleic acid was discovered from Lactobacillus plantarum AKU 1009a. Among the enzymes constituting the pathway, CLA-ER was identified as an enoate reductase, which can saturate the C=C bond in the 10-oxo-11(E)-octadecenoic acid. Based on the amino-acid sequence, CLA-ER is concluded to belong to the NADH oxidase/flavin reductase superfamily. The majority of proteins belonging to the NADH oxidase/flavin reductase superfamily were reported to have an NAD(P)H:FMN oxidoreductase activity and catalyze the reduction of flavins and nitroaromatic compounds.[3, 4] Some enzymes in this superfamily are also reported to take other activities. For example, IYD, an iodotyrosine deiodinase, salvages iodide from mono- and diiodotyrosine with a reduced FMN.[5] BluB, which is involved in the biosynthesis of vitamin B12, triggers the fragmentation and contraction of flavin mononucleotide and cleavage of ribityl tail to form DMB and D-erythrose 4-phosphate.[6] However, no enzyme has been reported to show an enoate reduction activity. Here, we report both the substrate-free and substrate-bound structures of CLA-ER, which elucidate the unique reaction mechanism and the structural basis of substrate specificity. Superposed structures of the two states revealed that a "cap" structure, formed by α5 and α6 helices, had a large conformational change upon the substrate binding. By the rotation of the "cap" structure, CLA-ER changed to its closed conformation and trapped its substrate tightly in an induced hydrophobic pocket. The carbonyl group of substrate was bound in the oxyanion hole formed by the amino group of Cys51 on the main chain and the hydroxyl group of FMN, which fixed the C=C bond adjacent to N5 position of FMN. Our structures also proposed that Cys51, a unique residue not conserved in the structure-determined NADH-oxidase/flavin reductase superfamily proteins, functioned as an electron donor for saturating the double bond of substrate together with FMN.